

WHAT IS CLAIMED:

1. A method for detecting a target nucleic acid molecule in a sample, said method comprising:

providing a first oligonucleotide primer coupled by a

5 linking agent to a solid substrate, wherein said first oligonucleotide primer is complementary to at least 18 contiguous nucleic acid residues of a first strand of a target nucleic acid molecule;

10 contacting the first oligonucleotide primer with the sample under conditions effective to permit any of the first strand of the target nucleic acid molecule present in the sample to hybridize to the first oligonucleotide primer;

15 extending the first oligonucleotide primer hybridized to the first strand of the target nucleic acid molecule under conditions effective to yield a double stranded extension product coupled by the linking agent to the solid substrate, wherein the linking agent is configured to position the first oligonucleotide primer sufficiently apart from the solid substrate to permit said extending;

20 denaturing the extension product under conditions effective to yield an immobilized extension portion complementary to the target nucleic acid molecule;

25 contacting the immobilized extension portion with a detection probe, having a nucleotide sequence like that of the target nucleic acid molecule and a label, under conditions effective to permit the detection probe to hybridize specifically to the immobilized extension portion; and

30 detecting the label immobilized on the solid substrate, thereby indicating a presence or absence of the target nucleic acid molecule in the sample.

2. The method according to claim 1, wherein the target nucleic acid molecule is a gene locus of an organism having DNA as its genetic information.

3. The method according to claim 2, wherein the organism is selected from the group consisting of humans, animals, plants, fungi, bacteria, and viruses.

4. The method according to claim 1, wherein said method is used to detect infectious diseases caused by bacterial, viral, parasitic, and fungal infectious agents.

5. The method according to claim 4, wherein the infectious disease is caused by a bacteria selected from the group consisting of *Escherichia coli*, *Salmonella*, *Shigella*, *Klebsiella*, *Pseudomonas*, *Listeria monocytogenes*,
10 *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare*, *Yersinia*, *Francisella*, *Pasteurella*, *Brucella*, *Clostridia*, *Bordetella pertussis*, *Bacteroides*, *Staphylococcus aureus*, *Streptococcus pneumonia*, B-Hemolytic strep., *Corynebacteria*, *Legionella*, *Mycoplasma*, *Ureaplasma*, *Chlamydia*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Hemophilus influenza*, *Enterococcus faecalis*,
15 *Proteus vulgaris*, *Proteus mirabilis*, *Helicobacter pylori*, *Treponema palladium*, *Borrelia burgdorferi*, *Borrelia recurrentis*, *Rickettsial pathogens*, *Nocardia*, and *Actinomycetes*.

6. The method according to claim 4, wherein the infectious disease is caused by a fungal infectious agent selected from the group consisting of *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Paracoccidioides brasiliensis*, *Candida albicans*, *Aspergillus fumigatus*, *Phycomycetes*, *Sporothrix schenckii*, *Chromomycosis*, and *Maduromycosis*.

7. The method according to claim 4, wherein the infectious disease is caused by a viral infectious agent selected from the group consisting of human immunodeficiency virus, human T-cell lymphocytotropic virus, hepatitis viruses, Epstein-Barr Virus, cytomegalovirus, human papillomaviruses, orthomyxo viruses, paramyxo viruses, adenoviruses, corona viruses, rhabdo viruses, polio viruses, toga viruses, bunya viruses, arena viruses, rubella viruses,
25 and reo viruses.

TOP SECRET//COMINT

8. The method according to claim 4, wherein the infectious disease is caused by a parasitic infectious agent selected from the group consisting of *Plasmodium falciparum*, *Plasmodium malaria*, *Plasmodium vivax*, *Plasmodium ovale*, *Onchocerca volvulus*, *Leishmania*, *Trypanosoma* spp., *Schistosoma* spp.,
5 *Entamoeba histolytica*, *Cryptosporidium*, *Giardia* spp., *Trichomonas* spp.,
Balantidium coli, *Wuchereria bancrofti*, *Toxoplasma* spp., *Enterobius vermicularis*,
Ascaris lumbricoides, *Trichuris trichiura*, *Dracunculus medinensis*, trematodes,
Diphyllobothrium latum, *Taenia* spp., *Pneumocystis carinii*, and *Necator americanus*.
- 10 9. The method according to claim 1, wherein said method is used to detect genetic diseases.
10. The method according to claim 9, wherein the genetic disease has a known nucleotide sequence and is selected from the group consisting of 21 hydroxylase deficiency, cystic fibrosis, Fragile X Syndrome, Turner
15 Syndrome, Duchenne Muscular Dystrophy, Down Syndrome, heart disease, single gene diseases, HLA typing, phenylketonuria, sickle cell anemia, Tay-Sachs Syndrome, thalassemia, Klinefelter's Syndrome, Huntington's Disease, autoimmune diseases, lipidosis, obesity defects, hemophilia, inborn errors in metabolism, and diabetes.
- 20 11. The method according to claim 1, wherein said method is used to detect cancer having a known nucleotide sequence and involving oncogenes, tumor suppressor genes, or genes involved in DNA amplification, replication, recombination, or repair.
12. The method according to claim 11, wherein the cancer is associated with a gene selected from the group consisting of BRCA1 gene, p53
25 gene, *Familial polyposis coli*, Her2/Neu amplification, Bcr/Ab1, K-ras gene, human papillomavirus Types 16 and 18, leukemia, colon cancer, breast cancer, lung cancer, prostate cancer, brain tumors, central nervous system tumors, bladder tumors, melanomas, liver cancer, osteosarcoma and other bone cancers, testicular
30 and ovarian carcinomas, ENT tumors, and loss of heterozygosity.

13. The method according to claim 1, wherein said method is used for environmental monitoring, forensics, and food and feed industry monitoring.

14. The method according to claim 1, wherein the linking agent
5 does not include a nucleic acid.

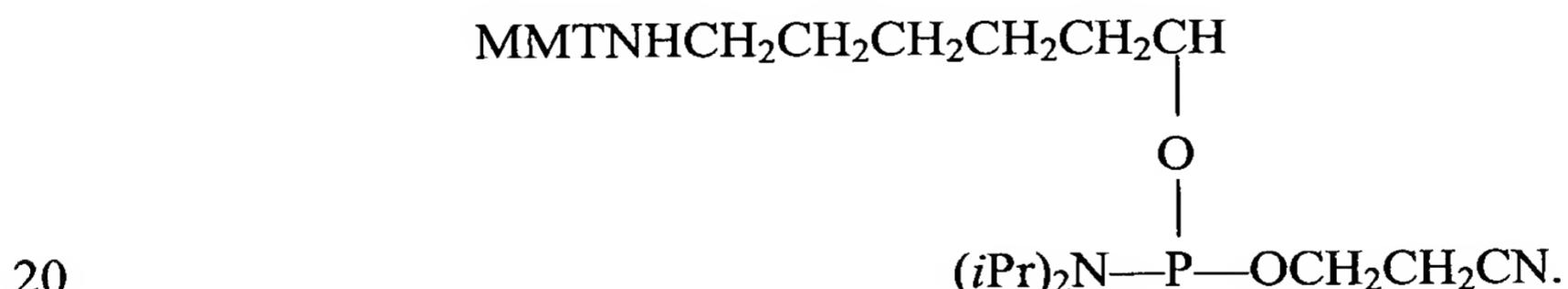
15. The method according to claim 1, wherein the linking agent has a length of about 5 to about 500 Ångstroms.

16. The method according to claim 15, wherein the linking agent has a length of about 25 to 250 Ångstroms.

10 17. The method according to claim 1, wherein said coupling of the first oligonucleotide primer with the linking agent is by a covalent bond.

18. The method according to claim 1, wherein said linking agent is generated by a 5'-Amino Modifier C6 spacer.

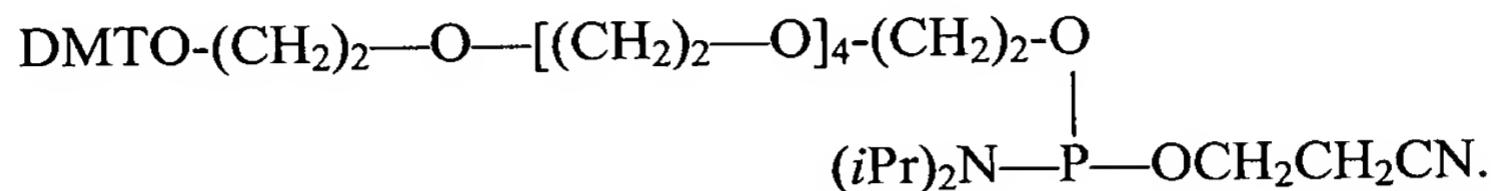
19. The method according to claim 18, wherein said 5'-Amino
15 Modifier C6 spacer comprises the following chemical structure:



20. The method according to claim 1, wherein said linking agent comprises a polyethylene glycol spacer.

21. The method according to claim 20, wherein said
25 polyethylene glycol spacer is selected from the group consisting of triethylene glycol spacers, hexaethylene glycol spacers, and heptaethylene glycol spacers.

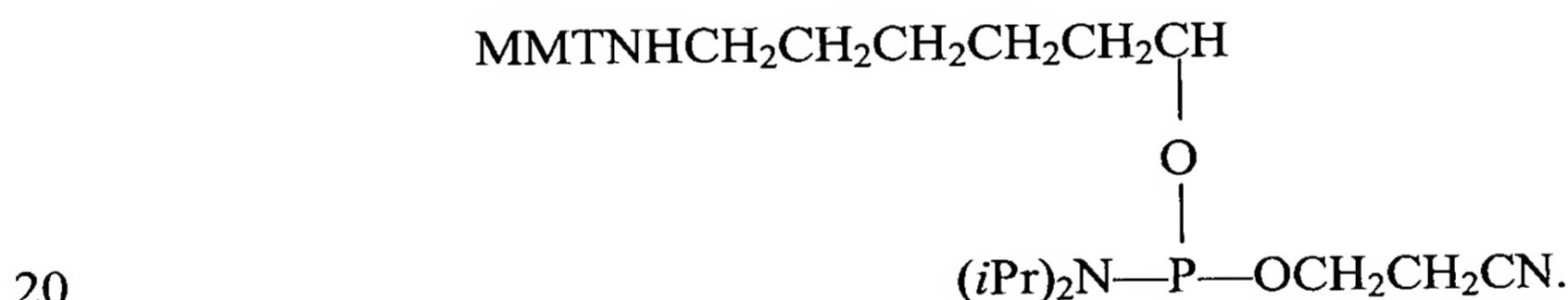
22. The method according to claim 21, wherein said hexaethylene glycol spacer is generated through the use of a Spacer Phosphoramidite 18 having the following structure:



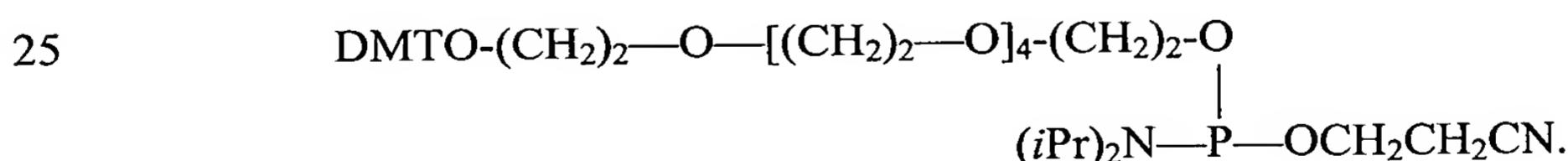
23. The method according to claim 22, wherein said Spacer Phosphoramidite 18 is used to introduce between about 1 to 20 hexaethylene glycol molecules into said hexaethylene glycol spacer.

24. The method according to claim 1, wherein said linking agent is generated using a 5'-Amino Modifier C6 spacer coupled to a polyethylene glycol spacer.

25. The method according to claim 24, wherein said 5'-Amino
15 Modifier C6 is of the formula:



26. The method according to claim 25, wherein said hexaethylene glycol spacer is generated through the use of Spacer Phosphoramidite 18 having the following formula:



27. The method according to claim 26, wherein said Spacer
30 Phosphoramidite 18 is used to generate between about 1 to 20 hexaethylene glycol
molecules.

28. The method according to claim 1, wherein the solid substrate is in a form selected from the group consisting of wells, microtiter plates, slides, discs, columns, beads, membranes, films, and composites thereof.

29. The method according to claim 28, wherein the solid substrate is functionalized with olefin, amino, hydroxyl, silanol, aldehyde, keto, halo, acyl halide, or carboxyl groups to permit attachment of the first oligonucleotide primer to the solid substrate.

5 30. The method according to claim 29, wherein the solid substrate is functionalized with an amino group by reaction with an amine compound selected from the group consisting of 3-aminopropyl triethoxysilane, 3-aminopropylmethyldiethoxysilane, 3-aminopropyl dimethylethoxysilane, 3-aminopropyl trimethoxysilane, N-(2-aminoethyl)-3-aminopropylmethyl 10 dimethoxysilane, N-(2-aminoethyl-3-aminopropyl) trimethoxysilane, aminophenyl trimethoxysilane, 4-aminobutyldimethyl methoxysilane, 4-aminobutyl triethoxysilane, aminoethylaminomethylphenethyl trimethoxysilane, and mixtures thereof.

15 31. The method according to claim 29, wherein the solid substrate is functionalized with an olefin-containing silane.

20 32. The method according to claim 31, wherein the olefin-containing silane is selected from the group consisting of 3-(trimethoxysilyl)propyl methacrylate, *N*-[3-(trimethoxysilyl)propyl]-*N'*-(4-vinylbenzyl)ethylenediamine, triethoxyvinylsilane, triethylvinylsilane, vinyltrichlorosilane, vinyltrimethoxysilane, vinyltrimethylsilane, and mixtures thereof.

25 33. The method according to claim 29, wherein the solid substrate is functionalized with a silanol polymerized with an olefin-containing monomer.

34. The method according to claim 33, wherein the olefin-containing monomer contains a functional group.

35. The method according to claim 33, wherein the olefin-containing monomer is selected from the group consisting of acrylic acid, methacrylic acid, vinylacetic acid, 4-vinylbenzoic acid, itaconic acid, allyl amine,

allylethylamine, 4-aminostyrene, 2-aminoethyl methacrylate, acryloyl chloride, methacryloyl chloride, chlorostyrene, dichlorostyrene, 4-hydroxystyrene, hydroxymethylstyrene, vinylbenzyl alcohol, allyl alcohol, 2-hydroxyethyl methacrylate, poly(ethylene glycol) methacrylate, and mixtures thereof.

- 5 36. The method according to claim 28, wherein the solid substrate is a polymer produced from a monomer selected from the group consisting of acrylic acid, methacrylic acid, vinylacetic acid, 4-vinylbenzoic acid, itaconic acid, allyl amine, allylethylamine, 4-aminostyrene, 2-aminoethyl methacrylate, acryloyl chloride, methacryloyl chloride, chlorostyrene,
- 10 dischlorostyrene, 4-hydroxystyrene, hydroxymethyl styrene, vinylbenzyl alcohol, allyl alcohol, 2-hydroxyethyl methacrylate, poly(ethylene glycol) methacrylate, and mixtures thereof, together with a monomer selected from the group consisting of acrylic acid, acrylamide, methacrylic acid, vinylacetic acid, 4-vinylbenzoic acid, itaconic acid, allyl amine, allylethylamine, 4-aminostyrene, 2-aminoethyl
- 15 methacrylate, acryloyl chloride, methacryloyl chloride, chlorostyrene, dichlorostyrene, 4-hydroxystyrene, hydroxymethyl styrene, vinylbenzyl alcohol, allyl alcohol, 2-hydroxyethyl methacrylate, poly(ethylene glycol) methacrylate, methyl acrylate, methyl methacrylate, ethyl acrylate, ethyl methacrylate, styrene, 1-vinylimidazole, 2-vinylpyridine, 4-vinylpyridine, divinylbenzene, ethylene
- 20 glycol dimethacrylate, *N,N'*-methylenediacrylamide, *N,N'*-phenylenediacrylamide, 3,5-bis(acryloylamido) benzoic acid, pentaerythritol triacrylate, trimethylolpropane trimethacrylate, pentaerytritol tetraacrylate, trimethylolpropane ethoxylate (14/3 EO/OH) triacrylate, trimethylolpropane ethoxylate (7/3 EO/OH) triacrylate, trimethylolpropane propoxylate (1 PO/OH) triacrylate, trimethylolpropane propoxylate (2 PO/OH) triacrylate, and mixtures thereof.

- 25 37. The method according to claim 28, wherein the solid substrate is a microwell suitable for use in quantitative assays that employ direct fluorescence detection.

38. The method according to claim 1, wherein said extending is carried out in an extension reaction mixture comprising dATP, dCTP, dTTP, dGTP, dITP, dUTP, and a polymerizing agent.

39. The method according to claim 38, wherein the
5 polymerizing agent is selected from the group consisting of *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *E. coli* DNA polymerase, T4 DNA polymerase, and *Pyrococcus* DNA polymerase.

40. The method according to claim 1, wherein the detection probe has a hybridization temperature of 20-85°C.

10 41. The method according to claim 1, wherein the label is selected from the group consisting of chromophores, fluorescent dyes, enzymes, antigens, heavy metals, magnetic probes, dyes, phosphorescent groups, radioactive materials, chemiluminescent moieties, electrochemical detecting moieties, and specific mass tags.

15 42. The method according to claim 41, wherein the label is a fluorescent dye selected from the group consisting of fluorescein, rhodamine, Texas Red, allophycocyanin, propidium iodide, Cy5, Cascade Blue, Dansyl, dialklyamino-coumarin, eosin, erythrosin, isosulfan blue, malachite green, Oregon green, pyrene, rhodamine green, rhodamine red, rhodol green, and derivatives of
20 these fluorescent dyes.

TOP SECRET - 100% DRAFT